PB84-197573

Teratogenic Evaluation and Fetal Deposition of Hexabromobenzene (HBB) and Hexafluorobenzene (HFB) in CD-1 Mice

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1984

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/J-84-013	2.	3. RE PB84-197573
4. TITLE AND SUBTITLE Teratogenic Evaluation and Fetal Deposition of Hexabromobenzene(HBB) and Hexafluorobenzene(HFB) in CD-1 Mice (Journal Version)		5. REPORT DATE 1984 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) K. Diane Courtney and James E. Andrews		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Inhalation Toxicology Division Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711		10. PROGRAM ELEMENT NO. ACAE1A 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development US Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Journal of Environmental Science and Health B19(1): 83-94, 1984

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified 20. SECURITY CLASS (This page) Unclassified	21. NO. OF PAGES 13 22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/J-84-013
JOURNAL ARTICLE

J. ENVIRON. SCI. HEALTH, B19(1), 83-94 (1984)

TERATOGENIC EVALUATION AND FETAL DEPOSITION OF HEXABROMOBENZENE (HBB) AND HEXAFLUOROBENZENE (HFB) IN CD-1 MICE

Key Words: Teratology, Mice, Hexabromobenzene (HBB), Hexafluorobenzene (HFB)

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ABSTRACT

HBB (hexabromobenzene) and HFB (hexafluorobenzene) were tested for their teratogenic potential in CD-1 mice. HBB and HFB were administered to pregnant mice from the 6th to the 16th day of gestation by gastric intubation. Neither HBB nor HFB were teratogenic or fetotoxic at doses up to 98.6 mg HBB/kg and 65.3 mg HFB/kg. No maternal toxicity was noted. HBB concentration in the fetuses indicated little, if any, accumulation. No HFB was detec-ed in the fetal or maternal tissues 24 hours after the last dose.

INTRODUCTION

The toxicity and bioaccumulation of organochlorine compounds is well established and has led to the curtailment of the manufacture and use of DDT and PCBs. During this time, other organohalides were being developed, the most notable being the introduction of PBBs into commerce. In addition, HBB (hexabromobenzene) has been used as a fire retardant (Pashin et al. 1974) and HFB (hexafluorobenzene) has been used as an anesthetic (Massey and Jackson, 1973; Hall and Jackson, 1973). HCB (hexachlorobenzene) is an industrial by-product and a lipophilic ubiquitous pollutant. Toxicity in human beings resulted from using grain treated with HCB for baking bread instead of its intended agricultural use. Additionally, there was a 95% mortality among the infants who were nursed by mothers who ate contaminated bread or who ate the bread themselves. These data have been summarized in a review (Courtney, 1979).

In teratology studies, HCB produced renal malformations and fetal toxicity with reduced fetal body weight in CD-1 mouse (Courtney et al., 1976). Placental or lactational transfer of HCB has been documented in human beings, mice and rats (Hall and Jackson, 1973; Courtney and Andrews, 1979). Since HCB was teratogenic in mice, HBB and HFB were tested for their teratogenic potential as well as for maternal and fetal deposition.

METHODS

Pregnant CD-1 mice were purchased with known insemination dates from Charles River Labs., Inc., Wilmington, MA. Detection

of vaginal plugs indicated day zero of gestation. Mice were housed 5 per cage in polycarbonate cages containing heat treated pine shavings (Betta Chips, North Eastern Products Inc., Warrensberg, N.Y.) and given feed (Wayne Lab Blox, Allied Mills, Inc, Chicago, Ill.) and filtered water (automatic watering system) ad libitum. An ambient temperature of 22±2°C, relative humidity of 55±5% and a 12-hr light-dark cycle were provided. Cages were housed under spun polyester filter bonnets. A sentinel mouse program monitored the animal holding rooms for the presence of rodent viruses, bacteria, and other pathogens. The HBB and HFB were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI, with purities of 98% and 99%, respectively.

HBB and HFB were administered daily by gastric intubation (IG) on the 6th through the 16th day of gestation. The doses of HBB were 19.4 and 98.6 mg/kg administered in 0.2 ml corn oil/ mouse/ day. These doses of HBB were equimolar to 10 and 50 mg HCB/kg which were used in the reported teratology studies.[6] The dose of 98.6 mg HBB/kg was in suspension in the corn oil. The doses of HFB were 6.5, 32.7, and 65.3 mg HFB/kg and were equimolar to doses of HCB of 10, 50, 100 mg HCB/kg. HFB was a liquid and readily miscible with the corn oil. The day 6 body weights of the dams were used to calculate the doses. All control mice received 0.2 ml/mouse/day of corn oil.

All mice were sacrificed on day 17, and the fetuses were weighed as a litter and examined. Each of two of the fetuses and each of their placentas from each litter were weighed individually

and frozen individually pending assay of each for HBB and HFB. The HBB and HFB were quantified using GLC with a nickel detector as described previously for HCB (Courtney et al, 1979). The extraction efficiency and quantification of HBB was the same as that of HCB. Of the remaining litter, half were stored in Bouin's solution until examined by dissection. The remaining fetuses were placed in 80% alcohol and stained with alizarin red S for skeletal examinations.

The following conventions were observed in compiling the data. If a fetus was either resorbed or dead, it was regarded as a dead fetus. Only live fetuses were examined for malformations. A live fetus was classified abnormal if it had at least one type of anomaly. To calculate the maternal weight gain, the difference in the maternal weight from day 6 to 17 was determined after subtracting the gravid uterus weight from the day 17 weight. The maternal liver to body weight ratio was determined using the maternal weight minus the gravid uterus weight.

All data are presented as the arithmetic means with standard errors of the means (SE) using the litter as the unit. The data were analyzed statistically using Chi square and ANOVA.

RESULTS AND DISCUSSION

Table 1 presents the toxicologic evaluation of the pregnant mice treated with HBB and HFB. HBB at a dose of 19.4 mg/kg produced a slight loss of maternal weight which was not statistically significant from the control group. The higher dose of HBB had no effect on the maternal weight gain. No other effects of HBB were

TABLE 1

Toxicologic Evaluation of Hexabromobenzene (HBB) and Hexafluorobenzene (HFB) in CD-1 Mice Treated Daily by IG on Days 6-16 of Gestation and Sacrificed on Day 17.

Compound	Dose mg/kg	No. Mice	Maternal wt. gain (g) $\bar{X}$ ± SE	Liver/Body wt. % $\bar{X}$ ± SE	Implants/ Litter $\bar{X}$ ± SE	Live Fetuses/ Litter $\bar{X}$ ± SE	Fetal wt. (g) $\bar{X}$ ± SE	Fetal Mortal. %
Corn Oil	a	6	3.62±0.52	6.53±0.27	12.8±0.5	11.8±0.8	0.97±0.07	8.03
HBB	19.4[b]	20	1.54±0.98	6.97±0.15	12.1±0.4	11.1±0.4	1.05±0.04	9.17
HBB	96.8[b]	21	3.43±0.43	7.06±0.12	11.9±0.3	10.7±0.4	1.07±0.02	10.57
Corn Oil	a	12	4.44±0.54	6.87±0.18	12.8±0.5	11.8±0.5	0.90±0.04	7.80
HFB	6.5	11	2.99±0.34	6.89±0.24	11.8±0.4	11.3±0.3	1.09±0.04	4.37
HFB	32.7	10	2.75±0.38	6.84±0.23	11.4±0.8	10.6±0.8	0.89±0.07	7.00
HFB	65.3	14	4.23±0.80	7.15±0.09	11.9±0.4	11.1±0.11	1.02±0.04	6.20

[a] 0.2 ml/mouse/day

[b] suspension in corn oil

noted on the maternal liver-to-body ratio, number of implantations per litter, number of live fetuses, fetal weight or fetal mortality.

HFB at the two lower dose levels produced a weight loss in the dams which was not statistically significant from the control group. No weight loss was noted at the highest dose level. No effects of treatment with HFB were seen on the dam's liver to body weight ratio, number of implantations per litter, number of live fetuses per litter, fetal weight or fetal mortality.

Table 2 presents the results of the examination of the fetuses for malformations. The corn oil control groups for both the HBB and HFB studies each had fetuses with enlarged renal pelves. Enlarged renal pelves were seen in all treatment groups. The incidence of this anomaly was comparable in all control and treated groups, and therefore is not considered to be a compound induced malformation. The only other malformation seen in the control mice was a fetus with partially ossified sternebrae. This was also seen in a fetus of the low dose group of HBB and is not regarded as a compound induced malformation.

Malformations such as small kidney, enlarged kidney, hydronephrosis, as well as the occurrence of distended and full urinary bladders were seen in groups treated with HBB or HFB. The incidence of each malformation in any one group was very low. However, since all of these malformations occurred in the urinary system, it seems that a low level of renal and possibly bladder malformations might be produced with these compounds. The incidence of each of the individual malformations was very low and not

TABLE 2

Malformations in Fetuses of Dams Treated with Hexabromobenzene (HBB) or Hexafluorobenzene (HFB) on Days 6-16 of Gestation and Sacrificed on Day 17 of Gestation.

Compound	Dose mg/kg	No. Studied (No. with Malformations) Litters	Fetuses	No. and Types of Malformations
Corn oil	a	6 (4)	71 (7)	6 enlarged renal pelves 1 sternebrae[b]
HBB	19.4	19 (7)	210 (10)	2 small kidneys 6 enlarged renal pelves 1 sternebrae[b] 1 supraoccipitals[b] 3 distended urinary bladders
HBB	96.9	21 (10)	222 (13)	9 enlarged renal pelves 1 exencephaly 1 encephalocoele 1 hydronephrosis 1 enlarged kidney 1 small kidney
Corn oil	a	12 (5)	144 (6)	6 enlarged renal pelves
HFB	6.5	11 (3)	124 (5)	5 enlarged renal pelves 1 small kidney
HFB	32.7	10 (5)	106 (6)	5 enlarged renal pelves 1 club foot
HFB	65.3	14 (6)	152 (8)	6 enlarged renal pelves 1 small kidney 1 distended urinary bladder

[a] 0.2ml/mouse/day
[b] reduced ossification

dose related and thus, these malformations cannot be considered a teratogenic effect of HBB or HFB. It should be noted that HCB at a dose of 100 mg/HCB/kg produced kidney anomalies in mice (Courtney et al, 1976).

The incidence of other malformations was very low and probably not a result of the treatment. There were no skeletal malformations other than the non-ossified or partially ossified sternebrae and supra-occipitals presented in Table 2.

The skeletons of the fetuses were evaluated for development by determining the number of calcified centers in the forepaws, hindpaws and sternebrae. In addition, the number of calcified ribs on each side of the fetus were counted and are presented as a rib profile in Table 3. There were no statistically significant differences in the number of bones calcified in the fetuses of the control group compared to those of the treated groups for HBB. Likewise, there were no differences in the number of bones calcified in the control fetuses and the HFB fetuses for the forepaw, hindpaw and sternebrae. The rib profile of both HBB and HFB treated fetuses did not differ significantly from the control profiles. Thus, neither HBB nor HFB were teratogenic in the CD-1 mice.

Table 4 presents the data on the concentration of HBB in the dam's whole blood, fat, placentas and fetuses. The tissues of the corn oil control group had zero residues of HBB. Two fetuses and their placentas of each litter were assayed. There was no difference in the concentration of HBB in the blood of the dams even though there was a five-fold difference in dose between the two groups. Since HBB at the highest dose was in suspension, it may not have been absorbed. However, it should be noted that similar studies with HCB in which the highest dose of 100 mg/kg was in suspension, there were dose related concentrations of HCB in the

TABLE 3

Skeletal Examination of Fetuses Exposed to Hexabromobenzene (HBB) or Hexafluorobenzene (HFB) on Days 6-16 and Examined on Day 17

Compound	Dose mg/kg	No. Litters	No. Fetuses	No. Calcified Bones, $\bar{X} \pm SE$ Forepaws	Hindpaws	Sternebrae
Corn oil	a	6	33	7.2±0.1	8.4±0.1	5.9±0.1
HBB	19.4	20	95	7.1±0.1	8.7±0.1	5.4±0.1
HBB	96.8	21	95	7.2±0.1	8.7±0.1	5.7±0.1
Corn Oil	a	12	68	7.1±0.1	8.7±0.1	5.7±0.1
HFB	6.5	11	54	6.9±0.1	8.7±0.1	5.6±0.1
HFB	32.7	10	40	5.8±0.7	7.1±0.3	4.6±0.3
HFB	65.3	14	60	6.7±0.1	8.3±0.1	5.3±0.1

Compound	Dose mg/kg	No. Litters	No. Fetuses	Rib Profile, %Fetuses with 12/12	12/13	13/13	13/14	14/14
Corn oil	a	6	33	0	0	90.0	6.7	3.3
HBB	19.4	20	95	0	0	93.0	6.1	1.0
HBB	96.8	21	95	0	0	89.1	8.9	2.5
Corn oil	a	12	68	0	0	82.3	7.3	10.4
HFB	6.5	11	54	0	0	81.8	5.5	12.7
HFB	32.7	10	40	0	4.0	71.3	10.7	14.0
HFB	65.3	14	60	0	0	86.7	4.4	8.9

[a] 0.2ml/mouse/day

HBB AND HFB IS CH-1 MICE

TABLE 4

Concentration of HBB in Fetuses and Placentas, as well as Blood and Fat Samples of the Dams[a]

HBB Dose	19.4 mg HBB/kg	96.8 mg HBB/kg[b]
No. Mice	19	21
Dam Whole blood, HBB-PPM	29.09± 2.20	28.63± 2.26
Dam Fat, HBB-PPM	260.48±20.30	289.97±31.11
Fetal body weight (g)	1.03± 0.04	1.07± 0.02
Fetal HBB-PPM	2.83± 0.16[c]	2.09± 0.20[c]
Placental weight (g)	0.13± 0.01[d]	0.15± 0.01[d]
Placental HBB-PPM	7.82± 0.79	4.72± 0.56

[a]All values are means ± SE

[b]High dose of HBB in suspension

[c]$p = < 0.05$, compared to each other

[d]$p = < 0.05$, compared to each other

maternal and fetal tissues (Courtney et al, 1979). Similarly, there was no difference in the concentration of HBB in the fat of the dams at these two dose levels. The concentration of HBB in the fetuses of the low dose group was significantly higher than that of the high dose group. The concentration of HBB in the placentas of the low dose group was slightly higher than that of the high dose group but the difference was not statistically significant. The concentration of HBB in the tissues does not reflect much accumulation, if any at all. The similarity of the

tissue concentrations of the two dose groups could be reflecting a two-stage depletion with a fast and slow rate as seen in HCB elimination. Other than that, there appears to be very little similarity of HBB to HCB in either transport across the intestines and placenta or in the accumulation. HCB readily accumulates in both maternal and fetal tissues while it appears that very little HBB is accumulated in the tissues and no dose related concentration of HBB could be determined.

A similar study on the accumulation of HFB in maternal and fetal tissues was performed. The tissues were taken 24 hours after the last dose and no HFB was detected in any of the tissues. Thus, HFB differs from both HCB which does accumulate and from HBB which accumulates very little based on equimolar doses administered to pregnant mice on days 6-16 of gestation. Although the three compounds are structurally similar with the only difference being the halide group, there are major differences in the toxicokinetics of the compounds.

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Received: July 20, 1983.